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TOXICITY STUDIES OF SELECTED CHEMICALS

TASK I:

THE DEVELOPMENTAL TOXICITY OF ETHYLENE DIBROMIDE INHALED BY RATS AND MICE DURING ORGANOGENESIS

APRIL 1976

FINAL REPORT

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15. SUPPLEMENTARY NOTES

This task was initiated to investigate the ability of Ethylene Dibromide to produce birth defects by the inhalation route.

16. ABSTRACT

The purpose of this study was to determine the teratogenic potential of Ethylene Dibromide (EDB) inhaled by rats and mice during organogenesis. This report describes the effects of Ethylene Dibromide (inhaled at a concentration of 32 ppm for 23 hr a day from gestational day 6 through 15) on fetal development.

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Final Report

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NOTICE

This report has been reviewed by the Office of Toxic Substances, EPA, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

SUMMARY

Ethylene dibromide (EDB) was administered at 32 ppm by inhalation to two experimental animal species for 23 hr/day (allowing 1 hr for servicing the animals and chambers) during organogenesis (day 6 through 15 of gestation). Charles River CD rats and CD-1 mice were used in these tests. In addition to the control, a third group of animals was used. These were given a reduced diet, but no EDB exposure.

In mice, it was observed that exposed animals consumed less feed and gained less weight than controls. Litter sizes were somewhat reduced as were weights, and a variety of skeletal anomalies involving incomplete ossification were noted. Because increased occurrence of similar phenomena was displayed by the reduced-diet, unexposed rats, it was determined that the defects were most likely attributable to malnourishment rather than to EDB exposure, *per se*.

In rats, a similar reduction in feed consumption and weight gain was seen. Similarly, litter size was somewhat reduced, but fetal weights were near normal. As was noted in mice, many of the observed defects could well be attributable to malnourishment rather than to EDB exposure, *per se*. However, an increase in fourth-ventricular hydrocephaly, reduction in the occurrence of fourteenth rib, and increase in the frequency of wavy ribs appear to be correlated to EDB exposure in this species.

I. INTRODUCTION

Ethylene dibromide (1,2-dibromoethane, EDB), is used as a scavenger in gasoline, a fumigant and a chemical intermediate. The estimated production of EDB was about 315 million pounds in 1972 and 331 million pounds in 1973.

The inhalation of EDB produced toxicity in experimental animals.[1] Rats exposed to a concentration of EDB in excess of 200 ppm died within 24 hr from respiratory or cardiovascular collapse. Mortality at concentrations less than 200 ppm were delayed and occurred sometimes as long as 12 days after treatment. During this time, rats lost weight, appeared rough and unkempt, became irritable and produced a bloody nasal discharge. Chronic inhalation studies (7 hr/day for 5 days/week for 6 months) indicated that rats, guinea pigs, rabbits and monkeys generally tolerated EDB at levels of 25 ppm. The results of these studies were used to establish a threshold limit value of 20 ppm for EDB by the American Conference of Governmental Industrial Hygienists.[2]

EDB exposure also produced a more insidious type of toxicity than previously described. A carcinogenic response was demonstrated by administering EDB orally to rats (40 and 80 mg/kg/day) and mice (60 and 120 mg/kg/day) five times a week.[3] This treatment produced a high incidence of gastric squamous cell carcinomas in both species as early as 10 weeks after treatment started. The tumors, which were originally located in the forestomach, metastasized throughout the abdominal cavity. In some animals the carcinoma migrated to the lungs and other tissues.[4] EDB was also shown to be mutagenic in bacteria[5] and Drosophila melanogaster.[6] EDB also affected spermatogenesis and the maturation of sperm in bulls,[7] damaged spermatogenic cells in rats[8] and reduced the egg weight of laying hens.[9] This type of toxicity is insidious because the continuous exposure to EDB can produce dramatic effects after a symptom-free latency period.

The present study was undertaken to evaluate the ability of EDB to produce this insidious type of toxicity. The inhalation route of exposure was selected because EDB is found in the atmosphere. The production of congenital defects was used as a measure of toxicity because development is a finely regulated process which is sensitive to disruption by many agents. These agents include both carcinogens and mutagens. In addition, these tests may be performed in a short period of time and are useful in identifying agents that may produce birth defects in humans.

II. METHODS

A. Animals

Charles River CD rats and CD-1 mice (Charles River Breeding Laboratories, North Wilmington, Massachusetts) were housed in our animal quarters for at least 7 days prior to use. The quarters were maintained at 72°C with a relative humidity of 50 ± 5% and a 7 AM to 7 PM photoperiod. Animals were given free access to powdered rodent chow (Wayne Lab-Blox, Allied Mills, Inc., Chicago, Illionis), and tap water except where indicated in the experimental protocol. During the treatment period, feed was changed daily in order to prevent possible accumulation of EDB in the feed.

B. Animal Exposure

Rochester type stainless steel chambers with a volume of about 3.5 m^3 were used in this study. Clean air at a flow rate of 10 to 12 changes per hr entered at the top of the chamber. EDB vapor was generated by bubbling nitrogen into a stainless-steel vessel which was maintained at 30°C. EDB entered the air stream upstream from the chamber. Mixing was initiated in a plenum at the top of the chamber and completed by two squirrel cage fans and a diffusion plate.

The EDB concentration in the chamber was monitored using gas chromatography and a flame ionization detector. EDB was resolved using a stainless steel column packed with 5% didecyl phthalate on 80/100 chromosorb and nitrogen (80 ml/min) as the carrier gas. The injection, column and detector temperatures were 160°C, 145°C and 170°C, respectively. Standards were prepared by serial dilutions of an EDB stock solution prepared in carbon tetrachloride.

C. Experimental Protocol

Female rats and mice were exposed overnight to proven male breeders. Successful mating (day 0 of gestation) was identified the next morning by the presence of sperm in vaginal smears from rats and copulation plugs in mice. Mated animals were divided into three groups: a control group, an EDB treated group and a feed restricted group. Animals were housed in the inhalation chambers for 10 days starting on day 6 of gestation. During this time the EDB treated group was exposed to 30 ppm of EDB for 23 hr a day. The remaining animals were housed under similar conditions; however, the EDB exposure was omitted.

Rats and mice were sacrificed on gestational day 20 or 18, respectively. A laparotomy was performed and the uterine horns were exposed. The umbilical cord was clamped and severed distally in order to prevent blood loss. Fetuses were removed, weighed and examined for external anomalies.[10] One-half of the fetuses from each litter were fixed in Bouin's solution and examined for soft-tissue anomalies by a free-hand slicing method.[10] The remaining fetuses were fixed in 70% alcohol, eviscerated, stored in 1% KOH and stained with alizarin red.[11] After differential decolorization, the skeletons were examined for anomalies.

D. Statistical Methods

Quantitative data, reported as the mean ± standard error, were initially analyzed by Bartlett's test for homogeneity.[12] The test of significance for homogeneous data was Dunnett's procedure.[12] In contrast, heterogeneous data were analyzed by the two-sample rank test.[13] For all tests the 0.05 level of significance was chosen except where indicated. The litter was considered to be the unit of observation.[14] All statistical tests, therefore, were based on the litter as the experimental unit.

III. RESULTS

Rats and mice inhaled EDB 23 hours a day for 10 days starting on day 6 of gestation. During this time, the average concentration of EDB was 31.6 ± 1.9 (mean ± S.E. for 36 determinations).

EDB exposure did not produce significant mortality in either rats or mice (Table 1). During the 10 day treatment period, rats and mice exposed to EDB consumed less feed and gained less weight than controls. After treatment, feed consumption of these animals returned to normal and they regained weight.

The various parameters of reproduction which were determined in this study are summarized in Table 2. EDB exposure was associated with a reduced litter size in rats. The fetal body weight was also reduced in mice exposed to EDB and mice whose feed consumption was limited during the treatment period. This effect on fetal weight was probably due to malnourishment during development rather than a direct toxicity of EDB on development.

The anomalies observed in fetuses from rats and mice are summarized in Tables 3 and 4, respectively. In rats, EDB exposure significantly increased the incidence of hydrocephaly of the fourth ventricle and of minor changes in rib development. In addition, one fetus had two shortened limbs with one ending in only four digits. These anomalies were not observed in fetuses from the rats with a restricted feed intake. Anomalies observed following exposure of mice to EDB included hydrocephaly of the third ventricle, hydrocephaly of the fourth ventricle, and variations in the normal ossification of the supraoccipital, incus and sternabrae. These anomalies were observed at a similar frequency in fetuses from mice exposed to EDB and from mice on a restricted feed intake.

TABLE 1

EFFECT OF ETHYLENE DIBROMIDE EXPOSURE DURING ORGANOGENESIS ON NUMBER, BODY WEIGHT CHANGE AND FEED CONSUMPTION OF RATS AND MICE

	ETHYLENE DIBROMIDE (ppm)		
	0[c]	31.6	0[d]
Rats			
Number			
Pregnant	18	18	17
Alive	18	18	17
Non-pregnant	0	10	1
Alive	0	10	1
Body Weight Change[a]			
During treatment	49 ± 5	-27 ± 4[e]	-46 ± 5[e]
After treatment	61 ± 5	80 ± 9	99 ± 4[e]
Feed Consumption[b]			
During treatment	21.1 ± 0.4	10.5 ± 0.6[e]	5.0 ± 0.1[e]
After treatment	30.8 ± 3.2	27.7 ± 1.2	29.7 ± 1.3
Mice			
Number			
Pregnant	17	13	9
Alive	17	13	7
Non-pregnant	7	15	11
Alive	7	14	11
Body Weight Change[a]			
During treatment	14 ± 1	1 ± 1[e]	5 ± 2[e]
After treatment	5.0 ± 0.9	6.2 ± 0.9	5.1 ± 1.7
Feed Consumption[b]			
During treatment	5.6 ± 0.2	3.5 ± 0.2[e]	3.6 ± 0.1[e]
After treatment	6.4 ± 1.6	6.4 ± 0.4	8.1 ± 0.2

a/ Gm/animal/interval for pregnant animals.
b/ Gm/animal/day for pregnant animals.
c/ Control group.
d/ Feed restricted to the amount indicated on this table.
e/ Significantly different from control (two-sample rank test).

TABLE 2

EFFECT OF ETHYLENE DIBROMIDE EXPOSURE DURING ORGANOGENESIS ON REPRODUCTION IN RATS AND MICE

	ETHYLENE DIBROMIDE (ppm)		
	0[a]	31.6	0[b]
Rats			
Pregnant survivors	18	18	17
Implants/dam	15.4 ± 0.3	12.4 ± 0.9[c]	15.2 ± 0.3
Viable fetuses (%)	99 ± 1	92 ± 6	99 ± 0
Dead fetuses (%)	0	0	0
Early resorptions (%)	1 ± 1	8 ± 6	1 ± 0
Late resorptions (%)	0	1 ± 1	0
Dams with complete resorptions	0	1	0
Live litters	18	17	17
Fetuses/dam	15.3 ± 0.3	12.2 ± 0.9[c]	15.1 ± 0.3
Males (%)	48 ± 3	59 ± 4	50 ± 3
Fetal weight (gm)	3.62 ± 0.04	3.53 ± 0.10	3.12 ± 0.06[c]
Mice			
Pregnant survivors	17	13	7
Implants/dam	10.3 ± 0.6	9.5 ± 0.8	8.6 ± 1.7
Viable fetuses (%)	83 ± 8	61 ± 11	42 ± 18
Dead fetuses (%)	0	0	0
Early resorptions (%)	15 ± 8	32 ± 11	46 ± 19
Late resorptions (%)	2 ± 1	7 ± 5	12 ± 12
Dams with complete resorptions	2	3	3
Live litters	15	10	4
Fetuses/dam	10.3 ± 0.4	8.2 ± 0.7	9.0 ± 2.3
Males (%)	50 ± 4	52 ± 6	54 ± 7
Fetal weight (gm)	1.24 ± 0.03	0.93 ± 0.06[c]	0.95 ± 0.09[c]

[a] Control group.
[b] Feed restricted to the amount indicated on Table 1.
[c] Significantly different from control (two-sample rank test).

TABLE 3

EFFECT OF ETHYLENE DIBROMIDE EXPOSURE DURING ORGANOGENESIS ON THE INCIDENCE OF ANOMALIES IN FETAL RATS

	Ethylene Dibromide		
Rat Fetuses	0[a]	31.6	0[b]
Inspected for			
External anomalies	269 (18)[c]	207 (17)	259 (17)
Soft tissue anomalies	129 (18)	99 (17)	116 (16)
Skeletal anomalies	140 (18)	108 (17)	135 (17)
External anomalies			
Limb reduction	0.0[d]	0.4 (1)	0.0
Soft tissue anomalies			
Hydrocephaly; lateral ventricles	0.0	0.9 (1)	1.0 (1)
third ventricle	0.0	1.6 (2)	0.0
fourth ventricle	6.9 (9)	17.8 (11)[e]	5.4 (4)
Hydronephrosis	7.3 (6)	9.7 (7)	0.0
Club foot	0.0	2.3 (2)	0.0
Skeletal anomalies			
Supraoccipital, incompletely ossified	1 (2)	12 (5)	3 (1)
Parietals, incompletely ossified	3.2 (6)	11.6 (7)	4.0 (2)
Fourteenth pair of ribs	14.9 (10)	2.4 (3)[f]	11.5 (8)
Wavy ribs	0.0	4 (4)[g]	0.0
Fused ribs	0.0	0.8 (1)	0.0

a/ Control group.
b/ Feed restricted to the amount indicated in Table 1.
c/ Total number of fetuses (total number of litters).
d/ Percentage of fetuses with the indicated anomaly calculated on a per litter basis. Number of litters affected is given in parenthesis.
e/ Significantly different from control group (two-sample rank test), ($P < 0.10$).
f/ Significantly different from control group (two-sample rank test), ($P < 0.05$).
g/ Frequency of litters affected significantly different from control group ($P < 0.05$) (Fisher exact probability test).

TABLE 4

EFFECT OF ETHYLENE DIBROMIDE EXPOSURE DURING ORGANOGENESIS ON THE INCIDENCE OF ANOMALIES IN FETAL MICE

	Ethylene Dibromide		
Mice Fetuses	0[a]	31.6	0[b]
Inspected for			
External anomalies	155 (15)[c]	81 (10)	36 (4)
Soft tissue anomalies	75 (15)	40 (10)	17 (4)
Skeletal anomalies	80 (15)	41 (10)	19 (4)
External anomalies			
Exencephaly	0.7 (1)[d]	0.0	0.0
Soft tissue anomalies			
Hydrocephaly: lateral ventricles	0.0	0.0	16.7 (1)
third ventricle	0.0	12.0 (3)	16.7 (1)
fourth ventricle	0.0	5.8 (2)	16.7 (1)
Skeletal anomalies			
Supraoccipital, incompletely ossified	7.2 (3)	52.2 (9)[g]	33.3 (2)
Incus, not ossified	5.4 (3)	56.2 (8)[g]	41.7 (2)
Sternabrae: incompletely ossified	4.7 (4)	22.3 (6)[f]	25.0 (3)[e]
not ossified	0.0	46.5 (7)[g]	41.5 (3)[f]
split	5.2 (3)	30.0 (5)	29.1 (3)

a/ Control group.
b/ Feed restricted to the amount indicated in Table 1.
c/ Total number of fetuses (total number of litters).
d/ Percentage of fetuses with the indicated anomaly calculated on a per litter basis. Number of litters affected is given in parenthesis.
e/ Significantly different from control group (two-sample rank test), ($P < 0.10$).
f/ Significantly different from control group (two-sample rank test), ($P < 0.05$).
g/ Significantly different from control group (two-sample rank test), ($P < 0.01$).

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